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# Some Experiments on $P^{32}$ : II. Chemical Procedure

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From the measurements, under various geometrical conditions, of activity of  $P^{32}$  contained in the sodium phosphate solution produced from the irradiated  $CS_2$  as described in Part II, we found that the amount of  $P^{32}$  obtained may be about  $1/15 \mu c$ .

The improvement of absolute yield of  $P^{32}$  and some physical studies are now in progress.

## 8. Some Experiments on $P^{32}$ .

### II. Chemical Procedure.

*Senji Utzino, Syuzo Akashi, Minoru Fukuda,  
Hiroaki Akagi and Tetsuya Hanai.*

The radioactive phosphorus  $P^{32}$  was prepared through irradiation of  $CS_2$  by means of radium-berillium neutron source. The nuclear reaction induced by the neutron bombardment is  $S^{32}(n, p)P^{32}$ . The chemical separation work of radioactive phosphate from irradiated  $CS_2$  was conducted in the following manner.

1. Extraction of  $P^{32}$  from  $CS_2$  as radioactive phosphoric acid, using nitric acid as oxidant and iodine as catalyser. —Into 7l. bottle with a glass-jointed reflux condenser are transferred 3.8l. of irradiated  $CS_2$  and as much as  $1/3$  volume of  $CS_2$  nitric acid having the same specific gravity as that of  $CS_2$ . The mixture is added with a small piece of iodine and bubbled 3 hours through aeration and then after the addition of small amount of water the supernatant nitric acid is separated by a separating funnel from  $CS_2$ .

2. Isolation of  $P^{32}$ - $H_3PO_4$  with carrier phosphate. —The separated nitric acid is distilled in 1l. retort until the residual volume becomes 2~3c.c. After the residue is transferred to a 50c.c. test tube and 0.3m mol of  $KH_2PO_4$  is added as carrier, the total phosphoric acid is precipitated by the addition of molybdic reagent as ammonium phosphomolybdate. This precipitate after being washed by ammonium nitrate solution is dissolved in ammonia and the phosphoric acid is reprecipitated as ammonium magnesium phosphate by the addition of magnesia mixture. The magnesium salt is washed several times with ammonia.

Conversion of insoluble ammonium magnesium phosphate into soluble sodium phosphate. —The magnesium salt is treated with stoichiometric quantities of sodium hydroxide with which it is allowed to react in a boiling water bath. The magnesium salt goes into solution as sodium phosphate and is separated from residual magnesia. The filtrate and washings of the insoluble magnesia are collected in a small casserol and adjusted to pH 7.2 with 0.1 *n* hydrochloric acid. The solution is evaporated to a small volume, filtered into a measured tube and the filtrate together with washings is filled up to 5c.c. The latter fluid being reserved as stock solution. The stock

solution has the osmotic pressure of about one half of the physiologic saline solution and is chemically composed of sodium phosphate in a ratio of  $\text{Na}_2\text{HPO}_4 : \text{NaH}_2\text{PO}_4 = 7:3$  with a small amount of sodium chloride produced by the procedure of pH adjustment. The *G-M* counter estimates that the stock solution contains about  $1/15 \mu\text{c}$ . For the biological tracer experiments described in the following report 0.5c.c. of the stock solution was injected subcutaneously to a mouse.

## 9. Some Experiments on $P^{32}$ .

### III. Biological Tracer Experiment.

*Katashi Inoue, Takehiko Kikuchi, Tadashi Miyake,  
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Of the radioactive phosphate stock solution, which had been prepared as stated above (I, II), 0.5c.c. lots were injected to male mice subcutaneously. The lot of the solution, which was administered to each mouse, contained  $P^{32}$  with the activity of about  $0.005 \mu\text{c}$  and the carrier  $P^{31}$  weighing 0.84 mg and of pH 7.2. Each two mice were killed every three, twelve and twenty-four hours respectively after the injection, and bones, liver, kidneys, muscles, testes, spleen, small intestine and blood plasm were removed from these animals, and the tissues were ashed, treated with dilute hydrochloric acid and desiccated. The measurement of  $P^{32}$  content in these desiccated samples with the *G-M* counter were carried out as mentioned above (I). One mouse was killed fourteen hours and twenty minutes after the injection, and the whole body tissues, the urine and the feces with the gastrointestinal contents of this animal were examined in the same manner as aforesaid.

The radioactive phosphorus in the tissues per unit weight was most abundant in bones, and in less quantities in liver and plasm, while small intestine, spleen, kidneys, testes and muscles contained minute amount of  $P^{32}$  per unit tissue weight.

The radiophosphorus content of bones, liver and plasm diminished rapidly, and that of testes slowly decreased, while in kidneys the labeled element was of the highest amount at twelve hours after the injection, while in other tissues the content of  $P^{32}$  did not particularly change with the lapse of time.

Estimating the whole weight of these tissues of one mouse, and calculating the  $P^{32}$  content in them, we found that the bulk of the radiophosphorus was in bones and muscles, while liver, plasm and small intestine contained only a small portion of the injected  $P^{32}$ , and in kidneys, testes and spleen the labeled element was in minute amount.

In fourteen hours and twenty minutes after the injection, ten and eleven per cent of the radiophosphorus was eliminated from the kidneys and the gastrointestinal tract respectively, when seventy-nine per cent of  $P^{32}$  was retained in the body tissue.